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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Stat5 is closely involved in mammary gland differentiation and lactation. We have previously shown that active Stat5 is lost during breast cancer progression and this loss is associated with a more aggressive disease status. In this study, we propose to investigate the ability of active Stat5 to induce differentiation as a means to suppress invasion and metastasis of breast cancer cell lines. Using a constitutively active Stat5a that is tyrosine phosphorylated and transcriptionally active in the absence of prolactin stimulation, we hypothesize that over expression of active Stat5 will correlate with increased expression of differentiation markers and reduced invasion in vitro and in vivo. We have generated two constitutively active Stat5a constructs, Stat5a-S710F and Stat5a-3ser, and have determined that Sta5a-3ser has a greater potential to be active in the absence of prolactin stimulation. We have generated lentiviral, adenoviral, and MDA-MB-231 stable cell lines expressing these constructs and are in the process of initiating both in vitro and in vivo differentiation and invasion studies outlined in the original proposal.

15. SUBJECT TERMS
Prolactin, Stat5a, breast cancer, differentiation, metastasis

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Introduction

The Prolactin (PRL)-Jak2-Stat5 pathway has been described as a key regulator in the normal growth, development, and differentiation of human breast epithelia. Recent evidence from our lab suggested that active Stat5 was a positive predictive marker of prognosis in breast cancer patients and the loss of active Stat5 correlated with a more aggressive disease state(1). Further, *in vitro* expression of Stat5 increased differentiation characteristics of human breast cancer cell lines(2). The specific aims of this proposal were designed to further investigate the role of Stat5 in breast cancer progression and metastasis. We hypothesize that active Stat5a suppresses invasion and metastasis of human breast cancer by promoting upregulation of differentiation markers, increasing homotypic adhesion, and inhibiting growth. We will test this hypothesis in human breast cancer cell lines both *in vitro* and *in vivo* using a novel constitutively active Stat5a construct.

Body

To facilitate investigation of the aims proposed, novel constitutively-active Stat5a mutants (ca-Stat5a) were designed to enhance the effects of Stat5a activation, particularly in breast cancer cell lines lacking appreciable PRL-Stat5 activation and signaling. Stat5a-S710F was previously described to be constitutively active in hematopoietic cell lines(3). Although we observed moderate constitutive activity of Stat5a-S710F in the absence of prolactin in human breast cancer cell lines, we attempted to identify a construct with a higher level of constitutive activity. Serine 725 and serine 779 have been previously reported by our lab to be inhibitory Stat5a serine phosphorylation sites and mutation of both Ser725 and Ser779 to alanine, resulted in a Stat5a construct with increased sensitivity to prolactin(4). We identified that combination of S710F with S725A and S779A (Stat5a-3ser) resulted in a construct with at least two times more constitutive activity than the S710F mutation alone (Figure 1) and was hyper-phosphorylated in response to prolactin compared to Stat5a-WT or Stat5a-S710F (Figure 2). Stat5a-3ser was constitutively phosphorylated in the absence of PRL-stimulation (Figure 3) and is more sensitive to low doses of PRL than Stat5a-WT (Figure 4).

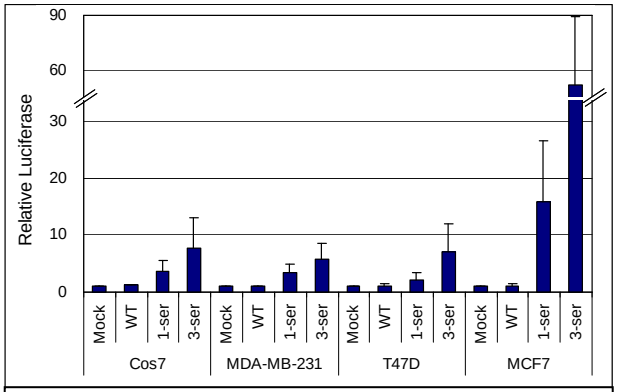

Figure 1. Stat5a-3ser has increased PRL-independent transcriptional activity compared to Stat5a-1ser (S710F). Four cell lines were transfected with Stat5a-WT, -1ser (S710F) or -3ser, hPRLR, and a Stat5a-repsonsive reporter construct. Cells were serum-starved in the absence of PRL for 18-hours. Cell lysates were measured for luciferase expression and normalized to mock cells.

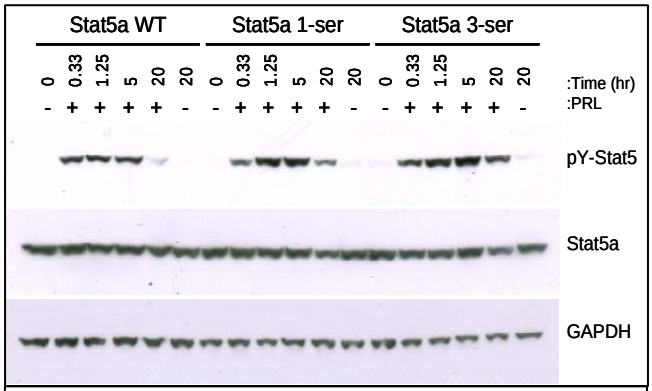

Figure 2. PRL induces more phosphorylation of Stat5a-3ser than Stat5a-WT or Stat5a-1ser (S710F). Cos-7 cells were transiently transfected with Stat5a-WT, 1ser (S710F), or 3ser and hPRLR. Following serum-starvation, cells were stimulated with PRL for the indicated times. Cell lysates probed for pStat5 expression.

We have generated Stat5a-WT and Stat5a-S710F adenovirus and are preparing to generate Stat5a-3ser adenovirus to use in the differentiation and invasion studies described in the proposal. In addition, T47D human breast cancer cells were created to stably express a tetracycline-responsive Stat5a-WT or Stat5a-S710F construct. We found standard transfection methods to result in low stable integration. As a result, we generated lentivirus expressing Stat5a-WT or Stat5a-3ser to facilitate higher rate of integration. Currently, we have prepared MDA-MB-231 human breast cancer cell lines stably expressing Stat5a-WT or Stat5a-3ser using the lentiviral system (Figure 5). We are in the process of initiating *in vivo* studies using Stat5a-WT and Stat5a-3ser MDA-MB231 stable cell lines.

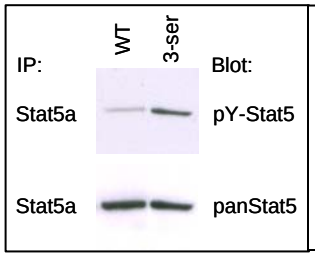

Figure 3. Stat5a-3ser is constitutively phosphorylated in the absence of PRL. MDA-MB-231 cells were transfected with Stat5a-WT or 3ser and serum-starved for 18-hr in the absence of PRL. Cell lysates were immunoprecipitated for Stat5a and immunoblotted for pStat5.

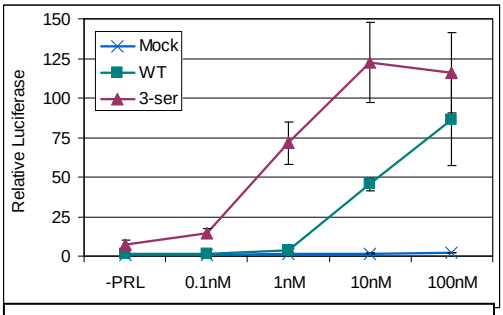

Figure 4. Stat5a-3ser is hyper-responsive to PRL. Cos-7 cells were transfected with Stat5a-WT or Stat5a-3ser, hPRLR, and a Stat5a-repsonsive reporter construct. Cells were serum-starved in the presence of increasing doses of PRL for 18-hr and cell lysates analyzed for luciferase expression.

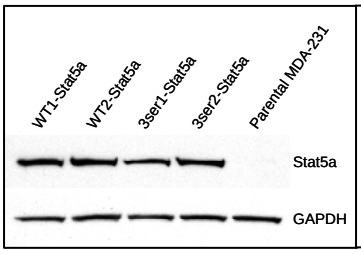

Figure 5. Stable Stat5a expression in MDA-MB-231. MDA-MB-231 were infected with lentivirus expressing indicated Stat5a construct.

Key Research Accomplishments

- Produced Stat5a-WT and Stat5a-S710F adenovirus for efficient gene delivery.
- Generated Stat5a-3ser, a ca-Stat5a construct containing three point mutations at Ser710, Ser725, and S779.
- Determined Stat5a-3ser phosphorylation and gene transcription was superior to Stat5a-S710F in the presence and absence of prolactin.
- Produced Stat5a-WT and Stat5a-3ser lentivirus to maximize integration and stable expression in breast cancer cell lines.
- Generated Stat5a-WT and Stat5a-3ser MDA-MB-231 stable cell lines.

Reportable Outcomes

Abstracts:

Ryder A, Utama FE, and Rui H. (2006) Characterization of a Constitutively Active Stat5a Mutant for use as a Tool to Induce Breast Cancer Differentiation and Reduce Invasion of Breast Cancer. Gordon Research Conference on Prolactin and Growth Hormone Family.

Conclusion

In summary, we have identified a constitutively active Stat5a construct that is tyrosine phosphorylated and transcriptionally active in the absence of prolactin stimulation. Work in progress and proposed experiments using Stat5a-3ser are designed to determine if reactivation of Stat5a can inhibit metastasis by inducing differentiation characteristics of human breast cancer cells. The outcomes of these experiments will greatly improve the knowledge base concerning the role of Stat5 in breast cancer. If our hypothesis is true, these data will also provide a foundation for research focused on developing differentiation therapies to treat breast cancer.

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Appendices

None.